საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 13, №4, 2019
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 13, no. 4, 2019

Parasitology and Helminthology

Identification of Potato Cyst Nematodes *(Globodera rostochiensis, Globodera pallida)* Spread in Kvemo Svaneti Region of Georgia

Dali Gaganidze[*, **], Nino Nazarashvili[*], Oleg Gorgadze[*], Ekaterine Abashidze[*], Mariam Aznarashvili[*, **], Eter Gvritishvili[*], Tinatin Sadunishvili[, §]**

** Laboratory of the Ministry of Agriculture, Tbilisi, Georgia*
*** Sergi Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, Tbilisi, Georgia*
*** § Academy Member, Georgian National Academy of Sciences, Tbilisi, Georgia*

ABSTRACT. Among the plant-pests that limit potato production and quality, the potato cyst nematodes (PCN) are the most harmful. PCN are among the most difficult plant pests to control. Cysts protected by the durable wall can survive for over 30 years. The aim of the present work is to carry out the field surveys of potato cyst nematodes *(Globodera rostochiensis, Globodera pallida)* in Kvemo Svaneti region of Georgia. Two forms of cystic nematodes were found in the soil samples. Their morphological and morphometric studies were carried out and the identified species were confirmed by the multiplex PCR. Analysis of PCR products confirmed that PCN in 9 samples from village Ghibi belongs to G. rostochiensis. *© 2019 Bull. Georg. Natl. Acad. Sci.*

Key words: *Globodera rostochiensis*, *Globodera pallida*, morphological and morphometric characteristics, identification, multiplex PCR

Among the plant-pests that limit potato production and quality the potato cyst nematodes (PCN) are the most harmful around the world. Yield losses caused by PCN are estimated up to 30% [1]. Two species of PCN – *Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenveber) Behrens – are recognized as plant quarantine pests and are included in EPPO A2 list [2]. Cyst nematodes live on the roots of host plants and can damage them causing growth retardation, water stress, nutrient deficiency, early withering and ultimately the yield loss [3].

PCN are among the most difficult plant pests to control. Cysts protected by a durable wall can survive for over 30 years [4]. Control measures for cyst nematodes are: use of healthy planting material, crop rotation, chemicals, solarization, bio-fumigation, weed removal. Currently, the most reliable control method against the cyst nematodes is breeding of resistant potato cultivars [5].

Control of PCN (*G. pallida* and *G. rostochiensis*) is regulated by Council Directive 2007/33/EEC, which establishes the measures against populations of PCN in order to determine their distribution and prevent their spread (Council Directive 2007/33/EC).

There was no legislative regulation of these pests in Georgia before 2016. By Resolution #302 from July 1, 2016, developed within the action plan of the DCFTA (Deep and Comprehensive Free Trade Area) the Government of Georgia established control over potato cyst nematodes (https://matsne.gov.ge/ka/document/view/3327972 ?publication=0). The Agreement about the legal acts approximation to EU legislation concerns the approval of rules of PCN control and research of these pests.

Taking into consideration the above mentioned, it is necessary to study distribution of PCN (*G. pallida* and *G. rostochiensis*) in the potato-growing areas of Georgia, which was never conducted.

The aim of this research is to conduct survey of potato cyst nematodes (*Globodera rostochiensis* and *G. Pallida*) in Kvemo Svaneti regions of Georgia and to identify PCN on the species level based on morphological – morphometric and molecular methods.

Materials and Methods

Soil and potato plant samples were collected according to EPPO protocols [2].

Soil samples were collected in each village, in a zig-zag pattern on the potato fields of the private sector using Metlitsky method [6]. Samples were collected also from infested potato plant roots; all samples were collected at 15-20 cm depth. To extract nematode cysts from the soil samples Fenwick can was used according to standard methods [2, 7]. Cysts were measured under stereoscopic microscope (Leica M50) by Bezooijen [8].

Morphological and morphometric studies were conducted on biological microscopes (Olympus Bx51). Permanent and temporary slides were prepared. Cyst was transferred to water drop; vulva and anus were removed using fine lancets; larvae and eggs were removed using brush and were transferred to glass slide with glycerin drop in paraffin ring. Slide was covered with coverslip and placed on moderately hot plate to melt paraffin and prepare permanent slides. To prepare temporary slides the larvae of the cysts were placed in a water drop on the glass plate.

Identification of the nematod species were carried out according to morphological and morphometric characteristics of the cysts and larvae using appropriate protocols [8]. Attention was paid to the shape and color of the cyst and the shape of the larvae stylet. For cysts the body length and width were measured. The number of cuticulare ridges and the Granek`s ratio were determined. In the case of the second stage larvae the length of the body, the length of the stylet, the width and shape of knob, the length of the hyaline region and tail were measured. The morphological-morphometric study of nematodes was carried out according to special protocols [9]. Statistical analysis was conducted using computer program "Anova" https://www.excel-easy.com/examples/anova.html.

Conventional PCR were used for molecular identification of PCN [2]. DNA isolation was conducted using the Nematode DNA extraction & purification kits (Invitrogen) according to manufacturer's protocol. Multiplex PCR test [10] were used for molecular identification. As positive control, *G. rostochiensis* DNA and *G. pallida* DNA were applied provided by Central Institute for Supervising and Testing in Agriculture, Department of Diagnostic Laboratory Olumouc, the Czech Republic. The comparative analyses of PCR products were performed by gel-electrophoresis method [11].

Table. Morphometric characteristics of potato cyst nematodes *Globodera rostochiensis* found in Kvemo Svaneti region, village Ghibe, Georgia

Cyst \ n*	15	18	8	27	11	16	31	10	5	38	3	7
Bodi length	559±89 (459-680)	588±92 (522-749)	593±79 (527-732)	535±92 (447-689)	647±29 (595-692)	545±90 (552-686)	589±54 (593-690)	648±85 (509-694)	598±32 (475-684)	584±84 (533-778)	590±57 (591-689)	650±42 (589-699)
Width	495±81 (436-670)	519±65 (426-636)	585±55 (534-670)	445±63 (393-588)	535±52 (473-572	493±64 (480-595)	498±78 (445-622)	613±48 (486-672)	574±58 (399-619)	560±67 (545-674)	579±37 (444-668)	499±49 (479-594)
Length/Width	1.1±0.06 (1.1-1.3)	1.1±0.05) (1.1-1.2)	1.1±0.05 (1.0-1.1)	1.1±0.04 (1.1-1.5)	1.2±0.08 (1.1-1.3)	1.1±0.08 (1.1-1.2)	1.1±0.06 (1.1-1.2)	1.1±0.04 (1.0-1.2)	1.1±0.07 (1.1-1.2)	1.1±0.07 (1.1-1.2)	1.1±0.04 (1.1-1.5)	1.1±0.04 (1.0-1.2)
Distance from anus to fenestra	55±6 (52-74)	70±8 (48-85)	77±7 (65-85)	68±3 (61-92)	60±4 (51-71)	57±5 (52-66)	87±9 (81-96)	74±5 (62-86)	72±2 (66-88)	68±3 (46-87)	58±4 (50-74)	55±6 (52-74)
Fenestra diameter	19±1 (18-24)	21±2 (19-25)	22±6 (21-23)	21±0.7 (20-22)	22±0.3 (21-23)	21±1 (20-23)	21±3 (20-23)	20±8 (19-22)	19±5 (18-21)	22±7 (21-23)	21±1 (20-23)	19±1 (18-23)
Granek\`s ratio	3.0±0.1 (2.9-3.4)	3.3±0.3 (3.1-4.0)	3.2±0.07 (3.1-3.4)	3.0±2 (2.7-4.5)	3.9±0.5 (3.1-4.0)	4.0±0.6 (3.4-4.5)	4.2±0.6 (3.8-4.6)	3.5±1.1 (2.9-4.4)	4.0±0.9 (3.6-4.7)	3.6±0.5 (3.1-4.2)	3.9±0.8 (3.3-4.0)	4.2±0.6 (3.8-4.6)
Cuticular ridges	17-18	≥11-17	16-17	17-22	17-19	17-18	17-23	17-20	16-22	17-22	17-23	17-19
Body length	421±11 (405-442)	416±6 (400-455)	- -	- -	344±13 (330-359)	369±10 (360-379)	416±6 (401-420)	397±19 (367-415)	329±8 (316-346)	411±4 (398-450)	379±6 (356-400)	410±2 (398-462)
Stilet length	20	20-22	-	-	20-21	22-23	22-23	21-23	20-22	20-21	22-23	21-23
Knob width	3.5	3	-	-	3	4	3	4	3	3	3	4
Hialine length	22	19-22	-	-	24	18-22	21-23	19-28	20-22	21-23	18-22	21-22
Tail length	47±2 (45-51)	46±2 (42-48)	- -	- -	41±2 (39-44)	42±1.5 (40-44)	45±2 (44-49)	47±2.5 (42-49)	45±2.8 (42-50)	45±2 (42-48)	42±3 (42-48)	42±1.4 (44-48)

*n – number of cysts in samples.

Results and Discussion

Out of the collected 50 samples of potato cyst nematodes (PCN) the cysts of Globodera spp. were revealed in 12 samples collected in village Ghibi (Fig.1). Perianal region of *G. rostochiensis* cyst and head part of the second stage juvenile larvae (J2) are represented in Figs. 2 and 3. Morphometric data of the *Globodera rostochiensis* cysts and J2 are represented in the Table.

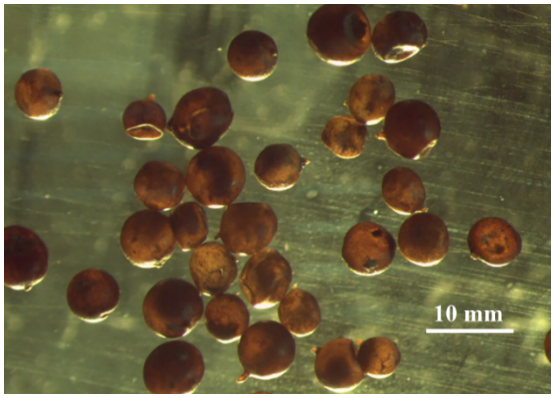

Fig. 1. Cysts of *G. rostochiensis.*

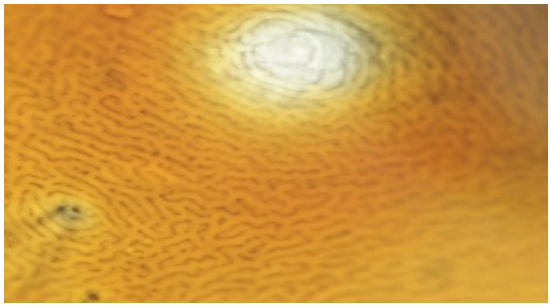

Fig. 2. Perianal region of *G. rostochiensis* cyst.

Fig. 3. Head part of the *G. rostochiensis* second stage juveline larvae.

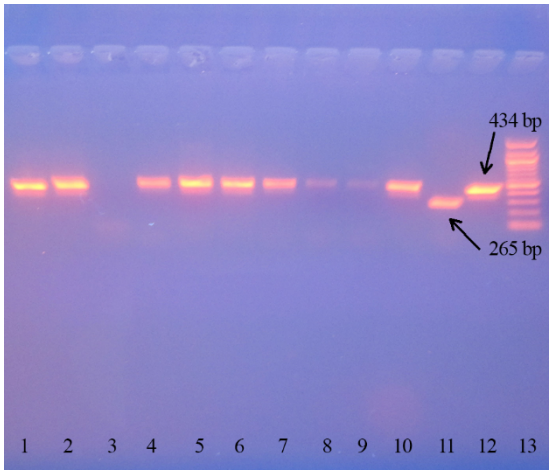

Fig. 4. Multiplex polymerase chain reaction (PCR) for *Globodera* sp. (*G. rostochiensis, G. pallida*) identification using primers PIp4, PIr3, and ITS. Lane 1, 2, 4, 5, 6, 7, 8, 9, 10 – *G. rostochiensis* from Kvemo Svaneti; 3-Negative control DNA (H_20); Lane 11-positive control – *G.rostochiensis* DNA; Lane 12-positive control *G.pallida* DNA; Lane 13-100 bp DNA ladder.

According to morphological and morphometric data, PCN is similar to G. rostochiensis; it has round shape and light-brown colour (Fig.1). Morphometric data concerning larvae also indicate similarity to *G. rostochiensis* (Table).

After morphological and morphometric analyses, multiplex PCR were conducted for *Globodera sp. species* (*G. rostochiensis* and *G. pallida*) identification. Electrophoresis of the PCR product on an agarose gel shows 434 bp band that confirms that PCN in 9 samples from Svaneti belongs to *G. rostochiensis* (Fig. 4).

Conclusion

As a conclusion, morphological, morphometric and molecular analyses of two forms of PCN found in investigated regions of Georgia show that one form of PCN belongs to *G. rostochiensis*.

The work was supported by Shota Rustaveli National Science Foundation of Georgia (Project # FR17-235/I).

პარაზიტოლოგია და ჰელმინთოლოგია

საქართველოს ქვემო სვანეთის რეგიონში გავრცელებული კარტოფილის ცისტიანი ნემატოდების (*Globodera rostochiensis, Globodera pallida*) იდენტიფიკაცია

დ. ღაღანიძე[*, **], ნ. ნაზარაშვილი[*], ო. გორგაძე[*], ე. აბაშიძე[*], მ. აზნარაშვილი[*, **], ე. გვრიტიშვილი[*], თ. სადუნიშვილი[**, §]

[*] საქართველოს სოფლის მეურნეობის სამინისტროს ლაბორატორია, თბილისი, საქართველო
[**] სერგი დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტი, საქართველოს აგრარული უნივერსიტეტი, თბილისი, საქართველო
*[**, §] აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია, თბილისი, საქართველო*

კარტოფილის იმ მავნებელებს შორის, რომლებიც ამცირებენ კარტოფილის პროდუქციას და ხარისხს, ცისტიანი ნემატოდები წარმოადგენენ საშიშ მავნებლებს. კარტოფილის ცისტიანი ნემატოდები ყველაზე რთულად გასანადგურებელ მავნებლებად ითვლებიან. მტკიცე გარსით დაცული ცისტები 30 წლის განმავლობაში ინარჩუნებენ სიცოცხლისუნარიანობას. წარმოდგენილი სამუშაოს მიზანს წარმოადგენდა საქართველოს ქვემო სვანეთის რეგიონში კარტოფილის ცისტიანი ნემატოდების (*G. rostochiensis, G. pallida*) გამოვლენა და იდენტიფიკაცია. ცისტიანი ნემატოდების 2 ფორმა იქნა აღმოჩენილი ნიადაგის ნიმუშებში. სახეობების იდენტიფიკაციისათვის ჩატარდა მორფოლოგიურ-მორფომეტრული კვლევები და შემდგომ მათი დადასტურება მოხდა მულტიპლექს პჯრ მეთოდით. პჯრ პროდუქტებით დადასტურდა, რომ სოფელ ღიბეში აღებულ 9 ნიმუშში გვხვდება *G. rostochiensis*. კარტოფილის ცისტიანი ნემატოდების სხვა ფორმის შესწავლა მოითხოვს შემდგომ კვლევას.

REFERENCES

1. Hodda M., Cook D.C. (2009) Economic impact from unrestricted spread of potato cyst nematodes in Australia. *Phytopathology*, **99**: 1387.
2. OEPP/EPPO (2013) PM 7/40 (3) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin*, **43**:119-138.
3. EFSA (2012) Scientific opinion on the risks to plant health posed by European versus non-European populations of the potato cyst nematodes *Globodera pallida* and *Globodera rostochiensis.* EFSA Panel on Plant Health (PLH). *EFSA Journal.,* **10**: 2644.
4. Winslow R.D., Wills R.J. (1972) Nematode diseases in potatoes. II. Potato cyst nematode, *Heterodera rostochiensis*, Webster (Ed), Economic Nematology 18. New York: Academic Press.
5. Trudgill D.L., Philips M.S., Alphey T.J.W. (1987) Integrated control of potato cyst nematode. *Outlook Agric*., **16**: 167.
6. Metlitskii O. Z. (1985) Ekologicheskie i tekhnologicheskie osnovy obnaruzheniia nematod. V: Printsipy i metody ekologicheskoi fitonematologii. E.L.Krall (Ed.), Petrozavodsk, 18-34 (in Russian).
7. Fenwick D.W. (1940) Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. *Journal of Helminthology,* **18**:155-172.
8. Van Bezooijen J. (2006) Methods and techniques for Nematology. Script, 112 p. - http://www.nem.wur.nl/NR/rdonlyres/CC0A519F-3ADD-4FFA-B473-959062BC9C7F/47004/MethodsandTechniquesforNematology.pdf [accessed on 1 Sept 2013].
9. OEPP/EPPO (2017) PM 7/40 (4) *Globodera rostochiensis* and *Globodera pallida*. *Bulletin OEPP/EPPO Bulletin*, **4**:174-197.
10. Bulman S.R., Marshall J.W. (1997) Differentiation of Australasian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR*). New Zealand Journal of Crop and Horticultural Science,* **25**:123-129.
11. Sambrook J., Russell D.W. (2001) Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Laboratory Press, Cold Spring Harbor.

Received June, 2019